PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| (51) International Patent Classification 5 : | | (11) Internati nal Publication Number: | WO 94/10328 |
|---|---|---|---|
| C12P 17/06 | A1 | (43) International Publication Date: | 11 May 1994 (11.05.94) |

(21) International Application Number: PCT/HU93/00051

(22) International Filing Date: 8 September 1993 (08.09.93)

(30) Priority data:
P 92 03458 4 November 1992 (04.11.92) HU

(71) Applicant *(for all designated States except US)*: BIOGAL GYÓGYSZERGYÁR RT [HU/HU]; Pallagi 13, H-4042 Debrecen (HU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : KÉRI, Vilmos [HU/HU]; Kemény Zs. 7, H-4028 Debrecen (HU). ILKŐY, Éva [HU/HU]; Munkácsi 37, H-1046 Budapest (HU). HŐGYE, Irma [HU/HU]; Egyetem 10, H-4027 Debrecen (HU). JEKKEL, Antónia [HU/HU]; Damjanich 38, H-1071 Budapest (HU). BAGDI, Ilona [HU/HU]; Kurucz 71, H-4025 Debrecen (HU). AMBRUS, Gábor [HU/HU]; Csalán 45/B, H-1025 Budapest (HU). JAKAB, Attila [HU/HU]; Sántha 10, H-4032 Debrecen (HU). ANDOR, Attila [HU/HU]; Peres 4, H-1221 Budapest (HU). DEÁK, Lajos [HU/HU]; Lehel 20, H-4032 Debrecen (HU). SZABÓ, István [HU/HU]; Reile 14, H-6000 Kecskemét (HU). BÁLINI, János [HU/HU]; Péchy 5, H-4032 Debrecen (HU). SHEIDL, Zsuzsanna [HU/HU]; Rózsa 51, H-1041 Budapest (HU). DELI, Etelka [HU/HU]; Jerikó 8, H-4032 Debrecen (HU). HORVÁTH, Gyula [HU/HU]; Kígyó 4-6, H-1051 Budapest (HU). SZABÓ, Csaba [HU/HU]; Fáy 21, H-4026 Debrecen (HU). LÁNG, Ildikó [HU/HU]; Izabella 13, H-1077 Budapest (HU). SZÉKELY, Imre [HU/HU]; Gyergyó 11, H-4028 Debrecen (HU). MORAVCSIK, Imre [HU/HU]; Mester 38, H-1095 Budapest (HU). KOVÁCS, Vera [HU/HU]; Civis 3, H-4032 Debrecen (HU). MÁTYÁS, Szabolcs [HU/HU]; Mária 34, H-1085 Budapest (HU). SZTÁRAY, Zsuzanna [HU/HU]; Sumen 28, H-4024 Debrecen (HU). ESZENYI, László [HU/HU]; Bekecs 14, H-4030 Debrecen (HU).

(74) Agent: INNOPATENT; Kartács 36, H-4032 Debrecen (HU).

(81) Designated States: AT, CA, DE, ES, US.

Published
*With international search report.*

(54) Title: PROCESS FOR THE ISOLATION AND PURIFICATION OF MEVINOLIN

(57) Abstract

The present invention relates to a process for the isolation of mevinolin by dissolving the active ingredient from the biomass into the fermentation liquor and subsequently separating it from the filtered fermentation liquor, which comprises carrying out the dissolution at a pH value between 7.5 and 10.0, preferably between 8.0 and 9.0, separating the active ingredient from the filtered liquor at a pH value between 4.5 and 1.0, preferably between 2.2 and 2.0, filtering and purifying it by methods known *per se*, preferably by recrystallization.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|---|---|---|---|---|---|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgystan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | | | SE | Sweden |
| CH | Switzerland | KR | Republic of Korea | SI | Slovenia |
| CI | Côte d'Ivoire | KZ | Kazakhstan | SK | Slovakia |
| CM | Cameroon | LI | Liechtenstein | SN | Senegal |
| CN | China | LK | Sri Lanka | TD | Chad |
| CS | Czechoslovakia | LU | Luxembourg | TG | Togo |
| CZ | Czech Republic | LV | Latvia | TJ | Tajikistan |
| DE | Germany | MC | Monaco | TT | Trinidad and Tobago |
| DK | Denmark | MD | Republic of Moldova | UA | Ukraine |
| ES | Spain | MG | Madagascar | US | United States of America |
| FI | Finland | ML | Mali | UZ | Uzbekistan |
| FR | France | MN | Mongolia | VN | Viet Nam |
| GA | Gabon | | | | |

# PROCESS FOR THE ISOLATION AND PURIFICATION OF MEVINOLIN

This invention relates to a process for the isolation and purification of mevinolin from fermentation liquor.

Mevinolin (lovastatin, monacolin K, MK 803) is a known antihypercholesterolemic agent, which can be produced by fermentation using either a microorganism belonging to the species Aspergillus terreus or different microorganisms identified as species belonging to the Monascus genus.

The isolation of the active ingredient is carried out either by extracting directly the fermentation liquor with a solvent or by extracting the filtered liquor and the biomass and subsequently purifying the crude product by chromatography.

For the extraction ethyl acetate, chloroform or benzene is used. The fermentation liquor contains partly the open-chain hydroxy acid form of mevinolin, that is 3,5-dihydroxy-7-[1,2,6,7,8,8a-hexahydro-2,6-dimethyl-8--(2-methylbutyryloxy)-naphtalene-1-yl]-heptanoic acid. This compound is heated in toluene to be lactonized to mevinolin. The purification of the crude product containing mevinolin exclusively in the form of lactone is carried out by chromatography and subsequent recrystallization (US patent specification No. 4,319,039, Hungarian patent specifications No. 182,069, 182,075 and 187,296).

According to US patent specifications Nos. 4,231,938 and 4,319,039 beside the extraction an $XAD_2$ adsorption resin is also used for the isolation of mevinolin.

The main disadvantage of the extraction method resides in the fact that the solvent dissolves, together with the active ingredient, a lot of concomitant contaminations rendering thereby the further purification more complicated and expensive. The purification at a proper efficiency can be accomplished namely by a multistage

column chromatographic method and subsequent re-crystallization.

Experiments have been carried out in order to compare the extraction method specified in Hungarian patent specification No. 187,296 to the method according to this invention for the isolation of mevinolin from fermentation liquor obtained by cultivation of an Aspergillus obscurus MV-1 holotype strain (deposition number: NCAIM (P)F 001189). The results of Example 1 prove that the product obtained from the fermentation liquor by extraction cannot be properly purified by recristalliztion. The preparation of a product suitable for pharmaceutical purposes requires further purification by column chromatographic methods.

The present invention aims at providing a process for the isolation of mevinolin from fermentation liquor which can be carried out more readily and more economically than the hitherto known processes and enables the preparation of the active ingredient in a quality suitable for pharmaceutical purposes.

The present invention is based on the recognition that the active ingredient can be separated at high efficiency directly from the filtrate of the fermentation liquor (hereinafter: filtered liquor) at a pH value between 4.5 and 1.0. The crude product separated in this manner does not require to be purified by chromatography, as only a surprisingly slight amount of contamination separates together with it. Thus a simple recrystallization is sufficient to obtain a product of suitable quality.

According to the process of the invention the active ingredient is dissolved from the biomass into the fermentation liquor at a pH value between 7.5 and 10.0, the biomass is filtered off, the crude product is separated from the filtered liquor at a pH value between 4.5 and 1.0 and purified by methods known per se, preferably by recrystallization.

The separation of the active ingredient has been investigated at different acidic pH values. The pH range of 2.4 to 1.8, especially 2.2 to 2.0 has been found to be

the most preferable. Besides, it has been found that the separation of the active ingredient from the filtered liquor, and especially the filterability of the precipitate can be improved by the addition of bivalent or trivalent metal salts, such as alkaline earth metal salts ($CaCl_2$, $MgCl_2$, $MgSO_4$) or earth metal salts [$(Al_2(SO_4)_3$].

In order to support what has been said in the following Table data are given to show the active ingredient content of the filtered liquor after filtering off the active ingredient at different pH values in the presence of or without adding calcium chloride to the filtered liquor. The content of the active ingredient was determined by HPLC.

| pH | Active ingredient content of the filtered liquor ( $g/cm^3$ ) | |
|---|---|---|
| | without adding any salt | in the presence of 0.2 M $CaCl_2$ |
| 7.0 | 418 | 60 |
| 6.0 | 387 | 65.8 |
| 5.0 | 201 | 103 |
| 4.0 | 58 | 50 |
| 3.0 | 31 | 22 |
| 2.0 | 14 | 10 |
| 1.5 | 10 | 10 |
| 1.0 | 8 | 8 |

Taking into consideration that the majority of the active ingredient is bound to the biomass, both the efficiency of the dissolution into the fermentation liquor and the amount of the concomitant contaminations are of great importance.

Besides, it has also been recognized that by carrying out the dissolution of the active ingredient into the

fermentation liquor at a pH value between 7.5 and 10.0, particularly between 8.0 and 9.0 both the loss of substance and the amount of the concomitant contaminations can be reduced to a minimum.

According to our experiences the dissolution of the active ingredient can be enhanced by adding a slight amount of additives to the mixture. Aliphatic alcohols having 1 to 4 carbon atom(s), glycols having 2 to 5 carbon atoms, secondary or tertiary amines having 1 to 3 carbon atom(s), alkyl acetates having 1 to 5 carbon atom(s), dimethyl-formamide, polyethylene glycol or polypropylene glycol may serve as additives.

In the following Table the active ingredient content of the filtered liquor is shown before the separation of the active ingredient at pH 9.0 and after the filtration thereof at pH 2.0 both in the presence of and without adding additives.

| Additive | Active ingredient content of the filtered liquor ($\mu g/cm^3$) | |
|---|---|---|
| 1 % by vol. | pH:9.0 | pH: 2.0 |
| Diethylamine | 412 | 9.2 |
| Triethylamine | 423 | 10.5 |
| Dimethylformamide | 460 | 6.9 |
| Methanol | 429 | 7.9 |
| Ethanol | 455 | 11.2 |
| Isopropanol | 467 | 8.7 |
| Ethylene glycol | 467 | 5.1 |
| Propylene glycol | 450 | 10.2 |
| Polypropylene glycol | 369 | 19.1 |
| Isobutyl acetate | 258 | 8.8 |
| Polyethylene glycol | 431 | 11.8 |
| Control (without additive) | 193 | 8.6 |

From the data of the above Table it can be established that upon the addition of different additives the active ingredient content of the filtered liquor is higher than without using such additives. So the additives promote the dissolution of the active ingredient from the biomass into the fermentation liquor. At the same time it can also be seen that the additives do not have an influence on the separation, this latter can be performed at the same efficiency either in the presence of or without adding additives. The addition thereof is optionally reasonable, as they render the technological procedure simpler. In the presence of additives namely a single formation of a suspension from the biomass is sufficient, while without using additives this procedure has to be repeated in order to achieve the same efficiency.

For the purpose of additive ethylene glycol and ethanol are particularly preferred.

According to our experiences the additives effect their favourable activity even when applied in as slight amount as 0.1 % by volume calculated upon the volume of the fermentation liquor, and even when applied in greater amounts they do not have an influence on the separation of the dissolved active ingredient.

| Concentration of ethanol % by volume | Active ingredient content of the filtered liquor ($\mu g/cm^3$) pH: 9.0 | pH: 2.0 |
|---|---|---|
| 0.1 | 400 | 8.9 |
| 0.5 | 425 | 8.5 |
| 1.0 | 455 | 11.2 |
| 5.0 | 447 | 11.5 |
| 10.0 | 441 | 13.0 |
| 15.0 | 434 | 18.1 |
| 20.0 | 430 | 26.0 |

The crude product can be purified by any known method,

e.g. by a simple recrystallization. According to our experiments it is preferable to carry out the re-crystallization from isobutyl acetate in such a manner that the solution of the substance in isobutyl acetate is washed with a weakly basic 2.5 w% ammonium sulfate solution adjusted to pH 8.5, the solvent phase is clarified with carbon, concentrated and the separated product is filtered off.

The advantages of the process according to the present invention are as follows: it renders possible the elimination of the extraction of both the fermentation liquor and the biomass from the technological procedure, the active ingredient separated from the filtered liquor at an acidic pH value is surprisingly pure, so it does not require to be purified by chromatography, but a simple recrystallization results in a product suitable for pharmaceutical purposes. Consequently the technological procedure is simple and can be accomplished economically, with a slight loss of substance (with a yield of higher than 90 %).

The process according to the invention can be applied by starting from any aqueous fermentation liquor cultured by a microorganism bio-synthetizing mevinolin either as the open-chain hydroxy acid or as lactone.

The invention is illustrated in detail by the following Examples of non-limiting character:

Example 1

Comparative experiment according to the extraction method specified in Hungarian patent specification No. 187,296

800 g of fermentation liquor cultured by an Aspergillus obscurus MV-1 holotype strain (deposition number: NCAIM (P)F 001189) containing a total amount of 670 mg of mevinolin both as lactone and as hydroxy acid were adjusted to pH 4 with 20 wt% sulfuric acid solution. The liquor was than extracted with 400 $cm^3$ of ethyl

acetate. The organic phase containing the active ingredient was separated and the aqueous residue was extracted again with further 400 $cm^3$ of ethyl acetate. The ethyl acetate extracts were combined (760 $cm^3$, active ingredient content: 643 mg), dried over anhydrous sodium sulfate and concentrated in vacuo. The concentrate was boiled in 100 $cm^3$ of toluene for 2 hours. Then the undissolved particles were filtered off and washed successively with 50 $cm^3$ of 5 wt% sodium hydrogen carbonate solution and
50 $cm^3$ of water. The toluene solution was dried over anhydrous sodium sulfate and evaporated in vacuo. The active ingredient content of the thus-obtained 3.5 g of oily product amounted to 630 mg. In order to crystallization the oily product was dissolved by warming in 15 $cm^3$ of ethanol and allowed to stand at a temperature of 5°C for 24 hours. The product did not separate in crystalline form. The solvent was then removed and the oily product (3.5 g) was devided into two parts.

1.75 g of product was recrystallized from 6 $cm^3$ of isobutyl acetate as specified in Example 2. The product did not separate in crystalline form.

The other portion of the product was subjected to column chromatography using a column filled with 20 g of Kieselgel 60 (0.063 to 0.2 mm) (height: 22 cm, diameter: 1.6 cm). The column was eluted with a 40:60 mixture of ethyl acetate and methylene chloride at a rate of 20 $cm^3$/hour. The 6 to 10 fractions containing the active ingredient were combined, clarified with activated carbon, filtered and evaporated in vacuo to yield 260 mg of yellowish white solid residue, which was recrystallized from ethanol. The separated crystals were filtered through a G-4 sieve, washed with 10 $cm^3$ of n-hexane and dried in vacuo at room temperature. Thus 180 mg of chromatographically pure mevinolin were obtained. The evaporation residue of the mother liquor obtained during the recrystallization was recrystallized again from ethanol to obtain further 35 mg of mevinolin. The quality

of the product was the same as that of the first generation.

Example 2

800 g of fermentation liquor cultured by an Aspergillus strain specified in Example 1 containing a total amount of 536 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1200 g with water. Then the solution was kept at a pH value between 8.5 and 9.0 with 20 wt% potassium hydroxide solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended twice in 400 $cm^3$ each of water. The suspension was adjusted to a pH value between 8.5 and 9.0 with 20 wt% potassium hydroxide solution, filtered again and the filtrates were combined. Thus 1900 $cm^3$ of filtered liquor containing 530 mg of active ingredient were obtained. The liquor was then adjusted to pH 2.1 with 15 wt% sulfuric acid solution, under stirring. The separated precipitate was settled, filtered, suspended in 100 $cm^3$ of a sulfuric acid solution adjusted to pH 2 and filtered again. The active ingredient concentration of the filtrate amounted to 12 $\mu g\backslash cm^3$.

The filtered aqueous precipitate was dissolved in 50 $cm^3$ of isobutyl acetate, the aqueous phase was separated and the solvent phase was concentrated to 2.5 $cm^3$. The concentrate was dissolved in 60 $cm^3$ of isobutyl acetate, washed twice with 60 $cm^3$ each of an aqueous ammonium sulfate solution adjusted to pH 8.5 with ammonium hydroxide, clarified with 0.5 g of carbon, concentrated to 10 $cm^3$, allowed to crystallize for 24 hours at 5°C, filtered and dried in vacuo. Thus 436 mg of mevinolin were isolated. Active ingredient content: 98.7 % (HPLC).

From the combined mother liquors further 65 mg of mevinolin were obtained in a purity of 92.8 %.

The crude products were combined and recrystallized from ethanol. Thus 450 mg of product were isolated.

Active ingredient content: 99.8 % (HPLC).

Dihydromevinolin content: 0.17% (GC)
$[\alpha]\ 25_D$= +329.8° (c=0.5; acetonitrile)

Example 3

800 g of fermentation liquor cultured by an Aspergillus strain specified in Example 1 containing a total amount of 605 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1200 g with water. Then 2,4 g of ethylene glycol were added to the mixture, and it was kept at a pH value between 8.5 and 9.0 by adding 20 wt% potassium hydroxide solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 400 $cm^3$ of water containing 0.8 g of ethylene glycol. The suspension was adjusted to a pH value between 8.5 and 9.0 with 20 wt% potassium hydroxide solution, filtered again and the filtrates were combined. The thus-obtained 1470 $cm^3$ of filtered liquor containing 600 mg of active ingredient were adjusted to pH 2.1 with 15 wt% phosphoric acid under stirring. The precipitate was settled for 4 hours. Further on the process specified in Example 2 was followed.
Thus 548 mg of mevinolin were isolated.
Active ingredient content: 99.7 % (HPLC).
Dihydromevinolin content: 0.15 % (GC)
$[\alpha]\ 25_D$= +329° (c=0.5; acetonitrile)

Example 4

800 g of fermentation liquor cultured by an Aspergillus strain specified in Example 1 containing a total amount of 575 mg of mevinolin both as lactone and as hydroxy acid were diluted to 1200 g with water. Then 2,4 g of ethylene glycol were added to the mixture, and the pH were kept at 9.0 to 9.5 by adding 20 wt% potassium hydroxide solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 400 $cm^3$ of water. The suspension was adjusted to a pH value between 9.0 and 9.5

with 20 wt% potassium hydroxide solution, filtered again and the filtrates were combined. Thus 1480 $cm^3$ of filtered liquor containing 567 mg of active ingredient were obtained. Then 3.5 g of calcium chloride were added to it and the solution was adjusted to pH 2.1 with 15 wt% sulfuric acid solution under stirring. The separated precipitate was settled for 4 hours. Further on the process specified in Example 2 was followed, with the difference that the active ingredient was dissolved from the precipitate with 120 $cm^3$ of isobutyl acetate.
Thus 527 mg of mevinolin were isolated.
Active ingredient content: 99.2 % (HPLC).
Dihydromevinolin content: 0.25% (GC)
$[\alpha]\ 25_D$= +329.5° (c=0.5; acetonitrile)

Example 5

10000 g of fermentation liquor cultured by an Aspergillus strain specified in Example 1 containing a total amount of 4180 mg of mevinolin both as lactone and as hydroxy acid were diluted to 15000 g with water. Then 30 g of ethylene glycol were added to the mixture, and it was kept at a pH value between 8.0 and 8.5 by adding 20 wt% potassium hydroxide solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 5 $dm^3$ of water containing 10 g of ethylene glycol. The suspension was adjusted to a pH value between 8.0 and 8.5 with 20 wt% potassium hydroxide solution, filtered again and the filtrates were combined. Thus 18200 $cm^3$ of filtered liquor containing 4091 mg of active ingredient were obtained. Then 20 g of magnesium sulfate were added to the mixture and it was adjusted to pH 2.1 with 15 wt% sulfuric acid solution, under stirring. The separated precipitate was settled, filtered, suspended in 1200 $cm^3$ of an aqueous sulfuric acid solution adjusted to pH 2 and filtered again. The filtered aqueous precipitate was dissolved in 600 $cm^3$ of isobutyl acetate, the aqueous phase was separated and the solvent phase was concentrated

to 30 $cm^3$. The concentrate was dissolved in 400 $cm^3$ of isobutyl acetate, washed twice with 400 $cm^3$ each of 2.5 wt% ammonium sulfate solution adjusted to pH 8.5 with ammonium hydroxide solution and clarified with 6 g of carbon by stirring for half an hour at room temperature. The solution was concentrated to 80 $cm^3$, allowed to crystallize for 24 hours at 5°C, filtered and dried in vacuo. Further on the process specified in Example 2 was followed.

Thus 3432 mg of mevinolin were isolated.
Active ingredient content: 99.1% (HPLC).
Dihydromevinolin content: 0.19 % (GC)
$[\alpha]\ 25_D$= +328.9° (c=0.5; acetonitrile)

Example 6

100 kg of fermentation liquor cultured by an Aspergillus strain specified in Example 1 containing a total amount of 44,3 g of mevinolin both as lactone and as hydroxy acid were diluted to 150 kg with water. Then 300 g of ethylene glycol were added to the mixture, and it was kept at a pH value between 8.5 and 9.0 by adding 20 wt% potassium hydroxide solution under continuous stirring for 2 hours. The biomass was then filtered off and suspended in 50 kg of water containing 100 g of ethylene glycol. The suspension was adjusted to a pH value between 8.5 and 9.0 with a 20 wt% potassium hydroxide solution, filtered again and the filtrates were combined. Thus 183 kg of filtered liquor containing 42.9 g of active ingredient were obtained. Then 200 g of magnesium sulfate were added to it and the solution was adjusted to pH 2.1 with 15 wt% sulfuric acid solution, under stirring. The separated precipitate was settled filtered, suspended in 12 $dm^3$ of a sulfuric acid solution adjusted to pH 2 and filtered again. The filtered aqueous precipitate was dissolved in 6 $dm^3$ of isobutyl acetate, the aqueous phase was separated and the solvent phase was concentrated to 300 $cm^3$. The concentrate was dissolved in 4 $dm^3$ of isobutyl acetate,

washed twice with 4 $dm^3$ each of 2.5 wt% ammonium sulfate solution adjusted to pH 8.5 with ammonium hydroxide solution and clarified with 60 g of carbon by stirring for half an hour at room temperature. The solution was concentrated to 0.8 $dm^3$, allowed to crystallize for 24 hours at 5°C, filtered and dried in vacuo. Further on the process according to Example 2 was followed.

Thus 37.03 g of mevinolin were isolated.

Active ingredient content:99.3% (HPLC).

Dihydromevinolin content: 0.18 % (GC)

$[\alpha]\ 25_D = +329.5°$ (c=0.5; acetonitrile)

What we claim is:

1. A process for the isolation of mevinolin by dissolving the active ingredient from the biomass into the fermentation liquor and subsequently separating it from the filtered fermentation liquor, which comprises carrying out the dissolution at a pH value between 7.5 and 10.0, preferably between 8.0 and 9.0, separating the active ingredient from the filtered liquor at a pH value between 4.5 and 1.0, preferably between 2.2 and 2.0, filtering and purifying it by methods known per se, preferably by recrystallization.

2. A process as claimed in claim 1, which comprises carrying out the dissolution in the presence of any of the following additive(s) applied in an amount of at least 0.1 wt% related to the volume of the fermentation liquor: aliphatic alcohols having 1 to 4 carbon atom(s), glycols having 2 to 5 carbon atoms, secondary or tertiary amines having 1 to 3 carbon atom(s), alkyl acetates having 1 to 5 carbon atom(s), dimethylformamide and/or polyethylene glycol and/or polypropylene glycol.

3. A process as claimed in claim 2, which comprises using as additive ethanol or ethylene glycol.

4. A process as claimed in any of claims 1 to 3, which comprises adding an alkaline earth metal salt or an earth metal salt to the filtered liquor prior to the separation.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/HU 93/00051

A. CLASSIFICATION OF SUBJECT MATTER

IPC[5]: C 12 P 17/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[5]: C 12 P 17/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim N. |
|---|---|---|
| A | DE, A1, 3 006 215 (SANKYO) 27 November 1980 (27.11.80), example 1. | 1 |
| A | DE, A1, 3 006 216 (SANKYO) 04 September 1980 (04.09.80), example. | 1 |
| A | Proceedings of the National Academy of Sciences of the United States of America, Volumne 77, no. 7., published July 1980 (Baltimore, USA), A.W. ALBERTS et al. "Mevindin: A highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent", pages 3957-3961, especially pages 3957, 3958. | 1 |

---

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 December 1993 (17.12.93) | 03 January 1994 (03.01.94) |
| Name and mailing address of the ISA/ AT<br>AUSTRIAN PATENT OFFICE<br>Kohlmarkt 8-10<br>A-1014 Vienna<br>Facsimile No. 1/53424/535 | Authorized officer<br>Wolf e.h.<br>Telephon N. 1/53424/133 |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/HU 93/00051

| Im Recherchenbericht angeführtes Patentdokument<br>Patent document cited in search report<br>Document de brevet cité dans le rapport de recherche | Datum der Veröffentlichung<br>Publication date<br>Date de publication | Mitglied(er) der Patentfamilie<br>Patent family member(s)<br>Membre(s) de la famille de brevets | Datum der Veröffentlichung<br>Publication date<br>Date de publication |
|---|---|---|---|
| DE A1 3006215 | 27-11-80 | AT A 1482/80 | 15-04-83 |
| | | AT B 372975 | 12-12-83 |
| | | AU A1 56678/80 | 13-11-80 |
| | | AU B2 534647 | 09-02-84 |
| | | BE A1 882325 | 19-09-80 |
| | | CA A1 1129795 | 17-08-82 |
| | | CH A 645891 | 31-10-84 |
| | | DE C2 3006215 | 05-01-89 |
| | | DE C2 3051097 | 08-02-90 |
| | | DK A 731/80 | 12-11-80 |
| | | DK B 148807 | 07-10-85 |
| | | DK C 148807 | 28-04-86 |
| | | ES A1 489751 | 16-04-81 |
| | | ES A5 489751 | 13-05-81 |
| | | ES A1 8104409 | 01-07-81 |
| | | FR A1 2456141 | 05-12-80 |
| | | FR B1 2456141 | 18-11-83 |
| | | GB A1 2049664 | 31-12-80 |
| | | GB B2 2049664 | 12-01-83 |
| | | HU B 182075 | 28-12-83 |
| | | IE B 49749 | 11-12-85 |
| | | IT A0 8067445 | 21-03-80 |
| | | IT A 1133075 | 09-07-86 |
| | | JP A2 55150898 | 25-11-80 |
| | | NL A 8001697 | 13-11-80 |
| | | SE A 8001338 | 12-11-80 |
| | | SE A0 8701483 | 09-04-87 |
| | | SE A 8701483 | 10-10-88 |
| | | SE B 467975 | 12-10-92 |
| | | SE B 468482 | 25-01-93 |
| | | SE C 467975 | 18-02-93 |
| | | SE C 468482 | 19-05-93 |
| | | US A 4323648 | 06-04-82 |
| | | KR A 8302329 | 22-10-83 |
| DE A1 3006216 | 04-09-80 | AT A 929/80 | 15-07-83 |
| | | AT B 373915 | 12-03-84 |
| | | AU A1 55673/80 | 28-08-80 |
| | | AU B2 532626 | 06-10-83 |
| | | BE A1 881825 | 20-08-80 |
| | | CA A1 1129794 | 17-08-82 |
| | | CH A 645890 | 31-10-84 |
| | | DD C 154494 | 24-03-82 |
| | | DE C2 3006216 | 31-10-85 |
| | | DE C2 3051175 | 21-12-89 |
| | | DK A 730/80 | 21-08-80 |
| | | DK A 470/85 | 01-02-85 |
| | | DK A0 470/85 | 01-02-85 |
| | | DK B 149095 | 20-01-86 |
| | | DK C 149095 | 16-06-86 |
| | | DK A 218/89 | 18-01-89 |
| | | DK A0 218/89 | 18-01-89 |
| | | DK B 165990 | 22-02-93 |
| | | DK C 165990 | 26-07-93 |
| | | ES A1 488796 | 16-02-81 |
| | | ES A5 488796 | 13-03-81 |
| | | ES A1 8103171 | 16-05-81 |
| | | FI A 800506 | 21-08-80 |
| | | FI B 66427 | 29-06-84 |
| | | FI C 66427 | 10-10-84 |
| | | FR A1 2449685 | 19-09-80 |
| | | FR B1 2449685 | 28-06-85 |
| | | GB A1 2046737 | 19-11-80 |
| | | GB B2 2046737 | 12-01-83 |
| | | HU B 182069 | 28-12-83 |
| | | IE B 49743 | 11-12-85 |
| | | IT A0 8067262 | 20-02-80 |
| | | IT A 1175260 | 01-07-87 |
| | | JP A2 55111790 | 28-08-80 |
| | | JP B4 59025599 | 19-06-84 |

| | | | |
|---|---|---|---|
| MX | U | 6314 | 01-04-85 |
| NL | A | 8001041 | 22-08-80 |
| NO | A | 800451 | 21-08-80 |
| NO | B | 153974 | 17-03-86 |
| NO | C | 153974 | 25-06-86 |
| NZ | A | 192919 | 06-07-84 |
| PH | A | 15145 | 24-08-82 |
| PL | A1 | 222120 | 20-10-80 |
| PL | B1 | 124304 | 31-01-83 |
| SE | A | 8001339 | 21-08-80 |
| SE | B | 453301 | 25-01-88 |
| SE | C | 453301 | 06-04-89 |
| SG | A | 67/84 | 08-02-85 |
| SU | A1 | 969702 | 30-10-82 |
| SU | A3 | 1158048 | 23-05-85 |
| ZA | A | 8000962 | 25-03-81 |
| KR | A | 8302801 | 16-12-83 |